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Effects of *Galleria mellonella* cadavers infected with *Heterorhabditis bacteriophora* and *Steinernema rarum*, their macerates and dead infective juveniles on *Meloidogyne javanica* suppression

Efecto de cadáveres de *Galleria mellonella* infectados con *Heterorhabditis bacteriophora* y *Steinernema rarum*, sus macerados y juveniles infectantes muertos en la supresión de *Meloidogyne javanica*

Eleodoro Eduardo Del Valle [1, 3], Marcelo Edmundo Doucet [2, 3]

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ABSTRACT

Nematodes of the *Meloidogyne* genus affect to most of crops of an economic importance in Argentina. Researches related to new control strategies are needed to reduce the damage produced by these organisms. The objective of this work was to compare the effects of *Galleria mellonella* cadavers infected with the Argentine isolates *Heterorhabditis bacteriophora* Rama Caída and *Steinernema rarum* NOE, cadaver macerates and dead infective juveniles (IJs) on *M. javanica* suppression. Experiments were performed using 24-well plates and pepper plants grown in a growth chamber. The entomopathogenic nematodes-infected *G. mellonella* cadavers, their cadaver macerates and dead IJs were effective in suppressing *M. javanica* second-stage juveniles under laboratory conditions. The use of *H. bacteriophora*-infected cadavers caused a significant decrease in the number of galls and egg masses on pepper plants parasitized by *M. javanica*, in a growth-chamber.

RESUMEN

Nematodos del género *Meloidogyne* afectan a la mayoría de los cultivos de importancia económica en Argentina. Investigaciones relacionadas con nuevas estrategias de control son necesarias para reducir el daño ocasionado por estos organismos. El objetivo de este trabajo fue comparar el efecto de cadáveres de *Galleria mellonella* infectados con los aislados argentinos *Heterorhabditis bacteriophora* Rama Caída y *Steinernema rarum* NOE, sus macerados y juveniles infectantes (JIs) muertos en la supresión de *M. javanica*. Las experiencias fueron conducidas utilizando placas de cultivo de 24 pozos y plantas de pimiento que crecieron en cámara de crecimiento. Los cadáveres infectados, sus macerados y JIs muertos fueron efectivos en suprimir juveniles de segundo estadio de *M. javanica* en las experiencias de laboratorio. En cámara de crecimiento, el uso de cadáveres infectados con *H. bacteriophora* causó reducción en el número de agallas y masas de huevos en plantas de pimiento parasitadas por *M. javanica*.

1 Facultad de Ciencias Agrarias, Universidad Nacional del Litoral, Kreder 2805, C.P. 3080, Esperanza, Santa Fe, Argentina. edelvalle@fca.unl.edu.ar

2 Laboratorio de Nematología, Centro de Zoología Aplicada, Jardín Zoológico, Rondeau 798, C. P. 5000, Córdoba, Argentina.

3 Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina.

Keywords

entomopathogenic nematodes • biological control • *Heterorhabditis bacteriophora* • *Steinernema rarum* • *Meloidogyne javanica*

Palabras clave

nematodos entomopatógenos • control biológico • *Heterorhabditis bacteriophora* • *Steinernema rarum* • *Meloidogyne javanica*

INTRODUCTION

Plant parasitic nematodes of the *Meloidogyne* genus cause economic losses in most of vegetable crops in Argentina (1). There are not many studies related to control strategies of these organisms in this country and the existing ones are based on chemical applications of high toxicity products that are harmful for the human health and the environment. Dead infective juveniles (IJs) of entomopathogenic nematodes (EPNs) and infected cadaver macerates can be used as alternatives for the management of plant-parasitic nematodes. Dead IJs of *Steinernema feltiae* and *S. riobrave* suppressed penetration of *Meloidogyne incognita* into tomato roots in laboratory trials (3). Bioassays performed to assess the effect of dead IJs of *Heterorhabditis baujardi* LPP7*, H. bacteriophora* JPM4, *S. carpocapsae* All and *S. feltiae* SN on tomato plants inoculated with *M. mayaguensis* showed that EPN species reduced gall number in plants; however, that result was not obtained for *S. feltiae* in a subsequent trial (11). The use of infected cadaver macerates proved to be efficient in suppressing the foliar nematode *Aphelenchoides fragariae* (8).

Results in the use of IJs of EPNs to control plant-parasitic nematodes have been equivocal (10), therefore, alternative application methods need to be explored. The use of macerates to evaluate foliar parasitic nematode suppression has been investigated (8), however, no studies have been done on root parasitic nematodes. Dead IJs application for plant parasitic nematode control still remain poorly studied. The increase of knowledge about the feasibility of these application methods would be helpful to overcome formulation and storage problems associated with live IJs. The objective of this research was to compare the effects of the application of *G. mellonella* cadavers infected with the Argentine isolates *H. bacteriophora* (Rama Caída) and *S. rarum* (NOE), their macerates and dead IJs, on the suppression of *M. javanica* (Nematoda: Meloidogynidae).

MATERIALS AND METHODS

Heterorhabditis bacteriophora Rama Caída and *S. rarum* NOE were reared in last instar *G. mellonella* larvae following Woodring and Kaya (12); IJs were harvested from modified White traps (9). Infected cadavers were produced by exposing last instar of *G. mellonella* to 100 IJs in 90-mm Petri dishes lined with filter paper and incubated at 25 °C for 6 days.

The *M. javanica* population was obtained from Santa Fe (Argentina). Eggs were extracted from pepper roots according Hussey and Barker (6). Second-stage juveniles (J2) were obtained by incubating egg masses in a moist chamber at room temperature.

To study the effect of macerates of *G. mellonella* cadavers infected with *H. bacteriophora* and *S. rarum* on *M. javanica* J2, a laboratory experiment was conducted in 24-well plates. The macerate was prepared using a blender with 10 6-day-old cadavers in 100 ml of distilled water, following Jagdale and Grewal (8). Water suspension (0.5 ml) with 100 *M. javanica* J2 and an equivalent volume of macerate were placed in each well. Distilled water with J2 was used as control.

The influence of dead IJs was also assessed in 24-well plates. Dead IJs were obtained by autoclaving IJs of both species at 120 ºC for 30 min and then placed in the wells. Treatments consisted of 1 ml of distilled water containing 0 (control), 10, 100 and 1000 autoclaved IJs of each EPN species, plus 1 ml of distilled water containing 100 J2 in each well.

In both earlier experiments, ten replicates per treatment were arranged in a completely randomised design and maintained at 25 ºC. The entire experiments were conducted three times. Mortality of J2 was recorded at 2, 4 and 6 days after exposure. Mortality data were arcsine square-root transformed and pooled before being subjected to ANOVA. Means were compared using Tukey's HDS test at $P < 0.05$ probability.

Pepper plants (*Capsicum annuum*) were used in growth chamber experiments as an experimental model. Pepper seedlings were obtained from California Wonder cultivar seeds germinated in 120-cc plastic cups filled with equal proportions of substrate and sterile sand. Seedlings were inoculated *with 1 ml of a water suspension containing* 300 *M. javanica* eggs. Treatments consisted of the application of an intact cadaver of *G. mellonella* infected with either *H. bacteriophora* or *S. rarum*, 5 ml of cadaver macerate, and *H. bacteriophora* or *S. rarum* live and dead IJs at a rate of 25 IJs/cm^2. Macerates and dead IJs were obtained as previously explained and placed in holes made in the soil around the plant stem. One cadaver per pot, was buried 3 cm into the soil. All treatments were applied immediately after *M. javanica* inoculation; the control treatment contained only eggs.

Two months after *M. javanica* inoculation, total number of galls, egg masses and eggs were counted for each plant. The average temperature was 30 ± 2 °C; plants were watered as needed. There were 6 replicates per treatment arranged in a completely randomised design. The experiments were conducted three times. Pooled data were analysed using an ANOVA and means were compared with a Tukey's HDS test at $P < 0.05$ probability.

RESULTS AND DISCUSSION

The present work is the first report using Argentine entomopathogenic nematode isolates to assess plant-parasitic nematode suppression and the effect of cadaver macerates on *M. javanica***.** Mortality rates of J2 caused by macerates in the laboratory increased significantly in *H. bacteriophora* (F= 167.44 ; df= 2, 87; $P < 0.0001$) and *S. rarum* (F= 49.93 ; df= 2, 87; $P < 0.0001$) treatments with increasing exposure time.

The effects of treatments at each observation time showed significant differences at 2 (F= 437.38 ; df= 2, 87; $P < 0.0001$), 4 (F= 398.7 ; df= 2, 87; $P < 0.0001$) and 6 days (F= 1153.4 ; df= 2, 87; $P < 0.0001$) (figure 1, page 209). Dead IJs influenced *M. javanica* J2 survival (figure 2, page 209).

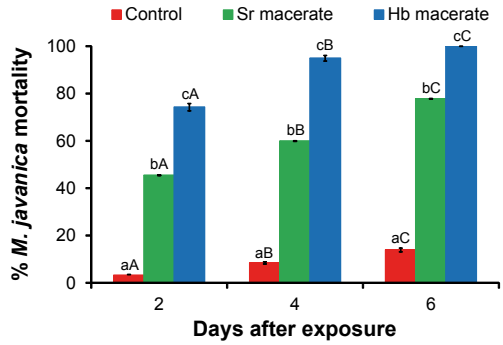
Mortality differed among concentrations, reaching high values with the application of 1000 dead IJs of both species. Nonetheless, *S. rarum* dead IJs caused greater suppression than *H. bacteriophora* dead IJs (2 days: F= 90.84; df= 6, 203; $P < 0.0001$; 4 days: F= 85.04; df= 6, 203; $P < 0.0001$ and 6 days: F= 97.29; df= 6, 203; $P < 0.0001$).

Antibiotic substances may have been responsible for repellent and/or toxic effects on plant-parasitic nematodes (3, 5). The secondary metabolites 3,5-dihydroxy-4-isopropylstilbene and indole obtained from the culture filtrate of *Photorhabdus luminescens* demonstrated nematicidal properties (5).

Differences were greater among doses and exposure times than between EPN species, although bacterium metabolites were qualitatively and quantitatively different between species and isolates (4, 2). Using the same methods employed in the present work, Jagdale and Grewal (8) evaluated mortality of *A. fragariae* caused by dead IJs and macerates of *S. carpocapsae*. They found that mortality of *A. fragariae* increased with increasing number of dead IJs applied and that it was high with macerates of *S. carpocapsae*-infected cadavers. Our results showed similar effects using *H. bacteriophora* and *S. rarum* against J2 of *M. javanica*.

The processes employed to obtain dead IJs used in the present work ensured death of symbiotic bacteria inside them. Suppression observed in the laboratory experiments was caused by the body of juveniles and/or the products of their decomposition and/ or of their dead symbiotic bacteria.

The suppressing effect indicated is consistent with findings reported by Jagdale *et al.* (7). In the present study, those metabolite effects were not observed when macerates and dead IJs were applied in pots. The results obtained could be negatively influenced by the high experimental temperature, which was used to simulate the regional crop conditions.

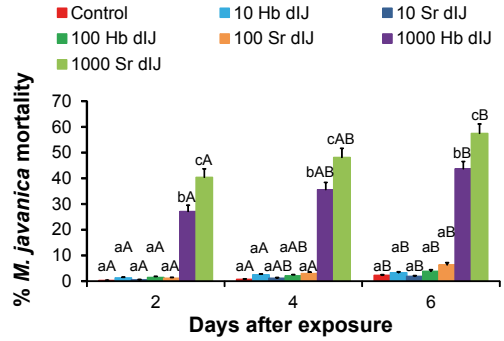


Bars with a different lower case letter indicate statistical differences in a single exposure time; bars with a different upper case letter indicate statistical differences among exposure times within a treatment, as determined by Tukey's test (P = 0.05).

Barras con letras minúsculas distintas indican diferencias estadísticas en un tiempo de exposición; barras con letras mayúsculas distintas indican diferencias entre tiempos de exposición en un tratamiento, de acuerdo con el test de Tukey (P = 0,05).

Figure 1. Mortality of *Meloidogyne javanica* second-stage juveniles caused by *Galleria mellonella* cadaver macerates infected with *Heterorhabditis bacteriophora* and *Steinernema rarum*.

Figura 1. Mortalidad de juveniles de segundo estadio de *Meloidogyne javanica* causada por macerados de cadáveres de *Galleria mellonella* infectados con *Heterorhabditis bacteriophora* y *Steinernema rarum*.



Bars with a different lower case letter indicate statistical differences in a single exposure time; bars with a different upper case letter indicate statistical differences among exposure times within a treatment, as determined by Tukey's test (P = 0.05).

Barras con letras minúsculas distintas indican diferencias estadísticas en un tiempo de exposición; barras con letras mayúsculas distintas indican diferencias entre tiempos de exposición en un tratamiento, de acuerdo con el test de Tukey (P = 0,05).

Figure 2. Mortality of *Meloidogyne javanica* second-stage juveniles caused by dead infective juveniles of *Heterorhabditis bacteriophora* and *Steinernema rarum* at different concentrations.

Figura 2. Mortalidad de juveniles de segundo estadio de *Meloidogyne javanica* causada por juveniles infectantes muertos de *Heterorhabditis bacteriophora* y *Steinernema rarum* en diferentes concentraciones.

There were statistical differences between the control and the *H. bacteriophora* cadaver treatment in the number of galls (*F*= 5.37; df= 4, 85; *P* = 0.0007) and egg masses (*F*= 5.05; df= 4, 85; *P* = 0.0011) (table 1), in the growth chamber. The suppression effect observed may be a consequence of bacterial metabolites and ammonia produced by insect cadavers (3). The four treatments, in which *S. rarum* was used, showed no differences in relation to the control (table 1). The present study was the first investigation to evaluate *S. rarum* for plant parasitic nematode control.

Table 1. Mean number and standard error of galls, egg masses and eggs of *Meloidogyne javanica* in pepper roots after treatment with infected *Galleria mellonella* cadavers, cadaver macerates, and dead and live infective juveniles of *Heterorhabditis bacteriophora* and *Steinernema rarum* in growth chamber experiments.

Tabla 1. Medias y error estándar de agallas, masas de huevos y huevos de *Meloidogyne javanica* en raíces de pimiento luego de ser tratados con cadáveres infectados de *Galleria mellonella*, sus macerados y juveniles infectantes vivos y muertos de *Heterorhabditis bacteriophora* y *Steinernema rarum* en experiencias desarrolladas en cámara de crecimiento.

Mean number of galls		
	H. bacteriophora	***S. rarum***
Control	25.17 ± 2.54 bc	25.17 ± 2.54 abc
Cadaver	15.17 ± 1.69 a	16.22 ± 1.60 a
Cadaver macerate	20.50 ± 2.05 ab	22.67 ± 1.75 ab
Dead juveniles	24.61 ± 2.94 abc	27.72 ± 2.79 bc
Infective juveniles	30.33 ± 2.79 c	34.39 ± 2.89 c
Mean number of egg masses		
	H. bacteriophora	***S. rarum***
Control	19.72 ± 2.10 b	19.72 ± 2.10 ab
Cadaver	8.94 ± 1.28 a	10.4 ± 1.35 a
Cadaver macerate	18.56 ± 2.49 b	19.83 ± 2.55 ab
Dead juveniles	21.67 ± 2.99 b	25.28 ± 3.65 b
Infective juveniles	21.78 ± 2.60 b	26.78 ± 3.67 b
Mean number of eggs		
	H. bacteriophora	***S. rarum***
Control	5276 ± 646 ab	5276 ± 646 ab
Cadaver	2782 ± 245 a	3467 ± 489 ab
Cadaver macerate	5398 ± 742 ab	6020 ± 809 ab
Dead juveniles	6471 ± 1013 b	7713 ± 1236 b
Infective juveniles	6267 ± 897 b	6971 ± 1118 ab

Means followed by different letters are statistically different as determined by Tukey's test (P = 0.05).

Medias seguidas de letras distintas son estadísticamente diferentes de acuerdo con el test de Tukey (P = 0,05).

CONCLUSION

Galleria mellonella infected cadaver macerates and dead IJs of *H. bacteriophora* and *S. rarum* suppressed *M. javanica* J2 in laboratory experiments. The use of *H. bacteriophora*-infected cadavers caused a significant decrease in the number of galls and egg masses on pepper plants parasitized by *M. javanica*. We confirmed the potential of infected *G. mellonella* cadavers for *M. javanica* control.

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